

Datasheet for ABIN1981847

STAT1,STAT3,STAT5 ELISA Kit

7 Images

7 Publications

[Go to Product page](#)

Overview

Quantity:	2 x 96 tests
Target:	STAT1,STAT3,STAT5
Binding Specificity:	pTyr694, pTyr701, pTyr705
Reactivity:	Human, Mouse, Rat
Method Type:	Cell ELISA
Application:	ELISA

Product Details

Purpose:	Cell-Based Human/Mouse/Rat Stat (1, 3, and 5) Phosphorylation ELISA Kit. Suitable for adherent whole cell lines.
Brand:	CellBIND®
Sample Type:	Cell Culture Cells
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	The antibodies provided in this kit recognizes human, mouse and rat STAT 1 phosphorylated at site Tyrosine-701, STAT 3 phosphorylated at site Tyrosine-705, and STAT 5 phosphorylated at site Tyrosine-694. This kit also recognizes total Stat 1, total Stat2 and total Stat 3 for comparison.
Characteristics:	<ul style="list-style-type: none">• Site and signal pathway-specific• In vitro detection of adherent cell culture• No sample lysis needed

Product Details

- Compatible with a standard ELISA plate reader
- Faster results than with ELISA
- Adaptable for high-throughput screening and drug discovery

Components:	<ul style="list-style-type: none">• uncoated 96-well Microplate• Wash Buffer A• Wash Buffer B• Fixing Solution• Quenching Buffer• Blocking Buffer• Anti-phospho antibodies• Anti-pan antibodies• HRP-Conjugated Secondary Antibody• TMB One-Step Substrate• Stop Solution
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Material not included:	<ul style="list-style-type: none">• Distilled or deionized water• 100 mL and 1 liter graduated cylinders• Tubes to prepare sample dilutions• Protease and Phosphatase inhibitors• Precision pipettes to deliver 2 µL to 1 mL volumes• Adjustable 1-25 mL pipettes for reagent preparation• Benchtop rocker or shaker• Microplate reader capable of measuring absorbance at 450 nm
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Target Details

Target:	STAT1,STAT3,STAT5
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Application Details

Plate:	Uncoated
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Protocol:	<ol style="list-style-type: none">1. Seed 10,000-30,000 cells into each well and incubate overnight.2. Apply various treatment, inhibitors or activators according to manufacture's instructions.3. Add 100 µL of Fixing Solution into each well and incubate for 20 min at RT with shaking.4. Add 200 µL of prepared 1X Quenching Buffer and incubate 20 min at RT.5. Add 200 µL of Blocking Solution and incubate for 1 h at 37 °C.6. Add 50 µL of 1X anti-phospho-protein specific antibody or anti-pan-protein specific antibody to each well and incubate for 2 h at RT.7. Add 50 µL of prepared 1X HRP-Anti-Rabbit or Mouse IgG and incubate for 1 h at RT.8. Add 100 µL of TMB One-Step Substrate Reagent to each well.9. Incubate 30 min at RT.
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10. Add 50 µL of Stop Solution to each well.
11. Read at 450 nm immediately.

Reagent Preparation:

NOTE: Thaw all reagents to room temperature immediately before use. If wash buffers contain visible crystals, warm to room temperature and mix gently until dissolved.

NOTE: Briefly centrifuge (~1,000g) ITEMS G, H, and I before opening to ensure maximum recovery.

For more information look at the picture.

Assay Procedure:

NOTE: ALL incubations and wash steps must be performed under gentle rocking or rotation (~1-2 cycles/sec).

1. Design your experiment. For example, see Figure 2 below.
OPTIONAL: If seeding HUVECs, HMEC-1 or other loosely attached cells, coat the Uncoated 96-Well Microplate (ITEM A) by adding 100 µL poly-L-Lysine (Recommended Sigma Aldrich, Cat#: P4832) into each well and then follow manufacturer's instructions. A pre-coated CellBIND® microplate or other poly-lysine treated tissue culture plate may be used in place of ITEM A.
2. Seed 100 µL of 20,000 to 30,000 cells into each well of the Uncoated 96- Well Microplate (ITEM A) provided and incubate overnight at 37 °C with 5 % CO₂.
NOTE: The optimal cell number used will vary on the cell line and the relative amount of protein phosphorylation. More or less cells may be used but this must be determined empirically.
NOTE: The cells can be starved ~4-24 hours (depending on cell line) prior to treatment with inhibitors or activators.
3. Apply various treatments, inhibitors (such as siRNA or chemicals) or activators according to manufacturer's instructions and incubate for the desired time points.
NOTE: It is recommended to dissolve inhibitors or activators into serum-free cell culture medium before treating the cells (unless otherwise stated in the manufacturer's instructions.)
4. Discard the cell culture medium by flipping the microplate upside down and gently tapping the bottom of the microplate over a sink.
5. Wash by pipetting 200 µL of the prepared 1X Wash Buffer A (ITEM B) into each well. Discard the wash buffer (same as step 4) and wash 2 more times for a total of 3 washes using fresh wash buffer each time. After the final wash, gently blot the microplate onto a paper towel to remove any excess/remaining buffer.
NOTE: To avoid cell loss, do not pipette directly onto the cells. Instead, gently dispense the liquid down the wall of cell culture wells. Also avoid the use of vacuum suction or too forcefully tapping the microplate when discarding any solution.
6. Add 100 µL of Fixing Solution (ITEM D) into each well and incubate for 20 minutes at room temperature.

Application Details

NOTE: The fixing solution is used to permeabilize the cells. 7. Repeat wash step 5.

8. Add 200 µL of the prepared 1X Quenching Buffer (ITEM E) into each well and incubate 20 minutes at room temperature.

NOTE: The quenching buffer is used to minimize the background response.

9. Wash 4 times with 1X Wash Buffer A.

10. Add 200 µL of the prepared 1X Blocking Buffer (ITEM F) into each well and incubate for 1 hour at 37 °C.

11. Wash 3 times with the prepared 1X Wash Buffer B (ITEM C).

NOTE: If needed, the microplate may be stored at -80 °C for several days after this wash.

12. Add 50 µL of the prepared 1X primary antibody (ITEM G1, G3, H1, or H3) into each corresponding well and incubate for 2 hours at room temperature. Incubate Items G2 and H2 overnight at 4 °C.

NOTE: Do not incubate Item G2 and H2 for 2 hours at room temperature or weak absorbances may result. Item G2 and H2 can be incubated overnight first followed by Item G1, G3, H1 or H3 incubations OR separate plates can be used.

13. Wash 4 times with 1X Wash Buffer B.

14. Add 50 µL of the prepared 1X HRP Conjugated secondary antibody (ITEM I) into each well and incubate for 1 hour at room temperature.

15. Repeat step 13.

16. Add 100 µL of the TMB Substrate (ITEM J) into each well and incubate for 30 minutes at room temperature in the dark.

17. Add 50 µL of the Stop Solution (ITEM K) into each well. Read at 450 nm immediately.

Restrictions:	For Research Use only
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Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
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Storage:	-20 °C
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Storage Comment:	The entire kit may be stored at -20°C for up to 6 months from the date of shipment. Avoid repeated freeze-thaw cycles.
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Expiry Date:	6 months
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Publications

Product cited in:	Brincks, Kucaba, Legge, Griffith: "Influenza-induced expression of functional tumor necrosis
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factor-related apoptosis-inducing ligand on human peripheral blood mononuclear cells." in: **Human immunology**, Vol. 69, Issue 10, pp. 634-46, (2008) ([PubMed](#)).

Janke, Witsch, Mages, Hutloff, Krocze: "Eminent role of ICOS costimulation for T cells interacting with plasmacytoid dendritic cells." in: **Immunology**, Vol. 118, Issue 3, pp. 353-60, (2006) ([PubMed](#)).

Coles, Wing, Smith, Coraddu, Greer, Taylor, Weetman, Hale, Chatterjee, Waldmann, Compston: "Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis." in: **Lancet (London, England)**, Vol. 354, Issue 9191, pp. 1691-5, (1999) ([PubMed](#)).

Caulfield, Fernandez, Sousa, Lane, Lee, Hawrylowicz: "Regulation of major histocompatibility complex class II antigens on human alveolar macrophages by granulocyte-macrophage colony-stimulating factor in the presence of glucocorticoids." in: **Immunology**, Vol. 98, Issue 1, pp. 104-10, (1999) ([PubMed](#)).

There are more publications referencing this product on: [Product page](#)

Images

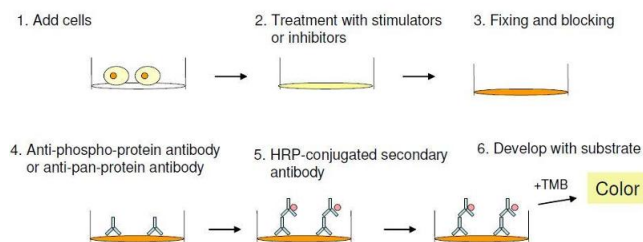
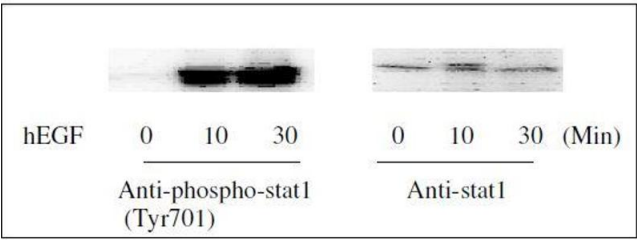


Image 1. Cell-Based protein phosphorylation procedure



Western Blotting

Image 2. Western blot analysis of extracts from 100 ng/mL hEGF treated A431 cells. Phospho-stat3 (Tyr705) and stat3 antibodies were LISA Kit used in both detection assays.

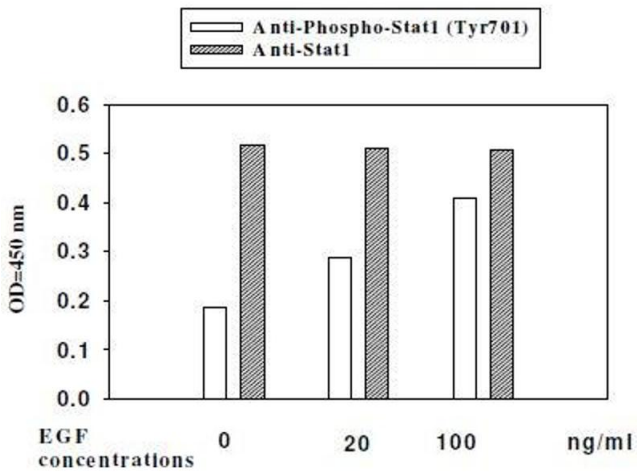


Image 3. A431 cells were stimulated by different concentration EGF for 30 min at 37 °C

Please check the [product details page](#) for more images. Overall 7 images are available for ABIN1981847.